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EXCYSTATION OF THE APOSTOMATOUS CILIATE, *HYALOPHYSA CHATTONI*, WITHOUT METAMORPHOSIS [1]

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Apostome ciliates are found encysted on the exoskeletons of Crustacea, excysting at the molt of their host (Chatton and Lwoff, 1935). A major group, the exuviotrophs, excyst and feed only on the fluid from the host's molt; while another large group, the histotrophs, also excyst at the death of an injured host and feed on the released body fluids. In either case the excystation of the encysted form (phoront) is preceded by an extensive metamorphosis. In the histotrophs the metamorphosis occurs soon after the migratory form settles on the host, but exuviotrophs metamorphose immediately before the ecdysis of the host. The result of this metamorphosis is a feeding stage (trophont) capable of engorging up to 30 times its initial volume (Bradbury and Trager, 1967b). After this rapid feeding, the apostome encysts and divides into numerous small ciliates (tomites) which seek out and settle on a new host.

Previous studies (Miyashita, 1933; Chatton and Lwoff, 1935; Trager, 1957; Bradbury and Trager, 1967b) suggest that the exuviotrophic phoronts are stimulated to metamorphose and excyst by the leakage of some compound or mixture of compounds that builds up in the host prior to ecdysis. Accordingly, the phoront stage of *Hyalophysa chattoni* found on brackish water shrimps was subjected to solutions of various substances that were likely to be concentrated in the shrimp just before ecdysis.

Materials and Methods

Grass shrimps (*Palaemonetes pugio* and *P. intermedius*) were collected near the Pamlico Sound Research Station, at Aurora, North Carolina. As many as several hundred were collected and held in two ten-gallon aquaria filled with 13.3‰ artificial sea water salts (Aquarium Systems Inc.), and equipped with aerater and filter. While the water temperature (22 to 25° C) and specific gravity (1.015) remained fairly constant, the pH varied between 6 and 8. The shrimps were fed daily. Of the 298 molting cycles recorded, 70% were 1 to 3 weeks long. All of the fresh molts contained *Hyalophysa chattoni* [identified by silver impregnation by the Chatton-Lwoff (Corliss, 1953) and the Protargol (Kirby, 1950) procedures].

Premolt shrimps were recognized by the gap that widens between the newly formed exoskeleton and the ends of the antennal scales and uropods as ecdysis nears (Passano, 1960). Metamorphosis in living *Hyalophysa* was recognized by

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crowding of food plaquettes to one side and the mid-ventral position of the contractile vacuole (Bradbury and Trager, 1967a).

Phoronts for *in vitro* experiments were obtained by cutting off a patch of exoskeleton containing numerous phoronts. On the shrimp, *Hyalophysa* preferentially settles in the cuticular depression at the base of the eyestalk. The exoskeleton of the living shrimp was cut from the base of the rostrum, medial to the base of the eyestalk beyond the field of phoronts. The second cut from the ventral edge of the carapace extended dorsally to intersect the first cut. The final cut was made parallel to the body surface just under the exoskeleton severing the musculature and connective tissue. These three incisions released a fragment that included the anterior end of the gill chamber, the antennule, the antenna—including the antennal scale, the eyestalk, and the base of the rostrum.

The fragment was washed vigorously in brackish water (13.3‰ artificial sea water with 500 units penicillin/ml and 0.05 mg streptomycin/ml (Bradbury and Trager, 1967b). The underlying muscle and connective tissue were picked away with forceps leaving only pieces of exoskeleton with the attached phoronts. During this half-hour procedure, the pieces were washed at frequent intervals to dilute the released body fluids.

All experimental solutions were made from the antibiotic brackish-water solution. All controls were in the antibiotic brackish water alone.

The cleaned pieces of exoskeleton were placed in 1 ml samples of solution in clear plastic disposable depression dishes (Scientific Products) and the entire dish was covered with "Parafilm" (American Can Company). A typical experiment would include 4 replicates of the control and 4 replicates of the experimental solution using phoronts from the same shrimp. The number of phoronts in each depression was counted and the percent of excystation was calculated by counting the remaining cysts at 4 hour intervals for 24 hours.

To obtain body fluid from uninfected *Palaemonetes*, the shrimp was crushed in about 0.5 ml of the antibiotic brackish-water solution. The fluid was collected in a hypodermic syringe and mixed with equal parts of the antibiotic brackish water.

All chemicals, except the ecdysterone, used to make experimental solutions were obtained from Sigma Chemical Company. The ecdysterone came from Mann Research Laboratories.

Results

A surprising observation in the course of these experiments was that the excysting phoronts on intermolt shrimps resemble in all visible respects the normal migratory stage, the tomite (Bradbury, 1966). No compound used in these experiments triggered metamorphosis of phoronts on intermolt shrimps. The phoronts were tested for excystation from 2 to 12 days after settling on the shrimps. In all experimental solutions, including 0.05 M Tris-HCl buffer (pH 9.0), 0.5% β-D glucose, 0.5% glycogen (from shellfish), 0.5% N-acetylglucosamine, and 10^{-7} M ecdysterone, the immerging ciliate had the body form and ciliary pattern of the tomite.

While the phoronts on intermolt shrimps always excysted as tomites, those on permolt shrimps excysted either as tomites or trophonts. In premolt shrimps

TABLE I

Averaged per cent excystation of trophonts and tomites from replicate experiments using 4 individual premolt shrimps*

Solutions	# Cysts	Hours					
		4	8	12	16	20	24
Control	156	51%	58%	83%	87%	87%	87%
I Glycogen (0.5)%	309	45	75	90	96	96	96
Control	234	21	69	90	90	90	90
II Glycogen (0.5%)	200	5	82	95	96	96	96
Control	111	13	39	97	99	99	99
III Glucose (0.5%)	182	12	22	98	100	100	100
Ecdysterone (10^{-7} M)	118	8	23	97	100	100	100
Control	48	45	61	82	87	87	87
IV Glucose (0.5%)	71	55	77	92	97	97	97
Glucosamine (0.5%)	88	55	69	85	91	93	93

* Per cent excystation was calculated after counting the remaining cysts. Only trophonts were found swimming in the dishes after 4 hours. Both trophonts and tomites were found after 8 hours. After 12 hours only tomites were found.

(Table I) phoronts excysted as trophonts within 4 hours and continued to excyst as trophonts for another 4 hours. But after 8 hours, the remaining phoronts excysted as tomites. Excystation of tomites from intermolt shrimps (Table II) started after an initial lag of 4 hours and increased rapidly to 80% or higher by 12 hours. Excystation was slower from 12 to 16 hours with little occurring after 16 hours. The general pattern of per cent excystation for both intermolt and premolt shrimps was the same after early differences. No significant effects on per cent excystation due to treatments alone were observed in any of the experiments. Transformation and analysis of variance of data for each shrimp were carried out using the Statistical Analysis System developed at NCSU for the use of the IBM 360/75 computer at Triangle Universities Computing Center.

In light of these unexpected results and because experiments using blood have resulted in excystation of trophonts from other crustaceae (Miyashita, 1933; Chatton and Lwoff, 1935; Trager, 1957; Bradbury and Trager, 1967b), shrimp body fluid was used as an experimental solution, with intermolt shrimps.

Experiment 1

Three live infested shrimps were placed in body fluid from other shrimps diluted 1:1 with antibiotic brackish water. No excystation was observed after 24 hours.

Experiment 2

Two fields of phoronts were cut from a shrimp 4 days from its last ecdysis. Neither piece was cleaned. One was placed in extra body fluids, while the other

was placed in the antibiotic brackish water alone. Excystation of tomites occurred from the first piece after 2 hours and from the second after 9 hours.

Experiment 3

One shrimp was found to have phoronts on the bristles of its maxillae. These phoronts excysted much more slowly than phoronts from the cuticular depression at the base of the eyestalk of the same shrimp. At 8 hours, 90% had excysted as tomites in the depression while none on the bristles excysted until after 8 hours (73% at 12 hours).

Experiment 4

When shrimps killed by stabbing near the field of phoronts were left in brackish water, no excystation of the phoronts occurred. Apparently they died on their hosts.

To test whether the excysted tomites would behave as normal tomites, they were offered uninfested shrimps as hosts. The tomites darted about over the shrimp's body as normal tomites would, but none of the excysted tomites were observed to re-encyst.

TABLE II

Averaged per cent excystation of tomites from replicate experiments using 6 individual intermolt shrimps

Solutions	# Cysts	Hours					
		4	8	12	16	20	24
Control	50	0	43%	81%	83%	88%	92%
I Glycogen (0.5%)	55	0	57	95	100	100	100
Control	96	0	94	100	100	100	100
II Glucosamine (0.5%)	108	0	87	96	98	98	98
Control	61	0	90	97	97	97	97
III Glycogen (0.5%)	33	0	63	97	97	97	97
pH 9.0 (0.05 M Tris-HCl buffer)	28	0	100	100	100	100	100
Control	26	0	29	58	69	74	84
IV Glycogen (0.5%)	20	0	25	50	90	90	90
pH 9.0 (0.05 M Tris-HCl buffer)	32	0	38	66	82	82	86
Control	120	0	61	85	89	91	91
V Glycogen (0.5%)	76	0	65	86	87	92	92
pH 9.0 (0.05 M Tris-HCl buffer)	71	0	79	87	88	88	94
Control	121	0	16	83	88	91	91
VI pH 9.0 (0.05 M Tris-HCl buffer)	79	0	11	63	79	82	82

Discussion

Because glycogen, glucose, and glucosamine markedly increase in Crustacea prior to ecdysis (Passano, 1960; Florkin, 1960), these compounds seemed likely to leak from the host just prior to the molt and perhaps thereby induce morphogenesis in the exuviotrophic apostome. Glucose and gycogen were also tested in antibotic brackish water buffered at pH 9.0, the pH of molting fluid (Dennell, 1960). None of these substances effected metamorphosis of *Hyalophysa* on shrimp although the same species on the hermit crab has been reported to metamorphose in weak solutions of glycogen (Bradbury and Trager, 1967b). On the hermit crab, phoronts are found primarly between gill lamellae within the gill chamber (Bradbury, 1966). The phoronts found on hermit crabs never have been observed to excyst as tomites. In view of this as well as the results of Cleveland and Nutting (1955) with the flagellates of the wood roach, Trager (1957) has suggested that molting hormone might effect the metamorphosis of exuviotrophs. Accordingly ecdysterone was tested for its effect on excystation with no observable results. Perhaps, as Trager has suggested, a series of events must occur to induce metamorphosis and excystation.

Two variables in all the experiments could not be completely controlled: (1) the amounts of body fluid in contact with phoronts during dissection, and (2) the amount of tactile stimulation the cysts received. Although the pieces of exoskeleton were washed repeatedly with clean solutions during the cleaning of the pieces, the phoronts still were bathed in body fluids for as long as one half hour. Perhaps Experiment #3 with phoronts encysted on bristles was the only experiment in which these two factors might not be significant. Although the phoronts on the bristles excysted after a longer lag period and did not excyst in as great numbers, excystation still occurred.

It should also be considered that all the conditions of habitat provided by the shrimp were not duplicated *in vitro*. Living shrimps always have some part of their anterior body in constant motion. Antennae and antennal scales move, the scaphognathite beats continuously, the maxillipeds and mouth parts are usually in motion. These movements are in addition to the usual walking and swimming movements of the shrimp. The effect of such movements is to bathe the phoronts in a constant through variable stream of water. It would seem that such a constant flow of water would provide the phoronts with a good supply of oxygen. Since phoronts of all apostome species whose life cycle is known settle on well aerated sections of the exoskeleton, the phoronts may have a relatively high oxygen requirement. They do move within the cyst and their contractile vacuoles are active. None of these experiments tested the effect of the experimental substances moving over the surface of the body (*i.e.*, phoronts bathed in a moving stream of a substance for a time before the current was stopped).

Since phoronts die *in situ* on intermolt shrimps (dead from any cause), the loss of currents of water in addition to failure to release body fluids until decomposition begins may explain why these phoronts do not excyst. Another possibility is that the lack of oxygen affects the phoronts before the body fluids leak from the shrimp.

In Experiment #1 (living shrimps in 1:1 brackish water and body fluids) no excystation occurred even though the two conditions of water currents and body

fluid were met. However, this experimental solution was cloudy and gummy with coagulated shrimp blood. Although antibiotics were added, bacterial action was just retarded—not stopped. The viscous water currents were unlikely to be comparable to water currents in the shrimp's natural environment. On the other hand, excystation may require both body fluids and cessation of movement. If this were so, the results of Experiments 2 and 3 would be expected because the large amount of body fluids in combination with a lack of water currents would lead to excystation. In Experiment #4 the stabbed shrimp would release body fluids, but they would be quickly diluted. The wound was behind the field of phoronts. This would mean that the flow of water would carry the body fluids away from the phoronts. The swift coagulation of shrimp blood would quickly stop any great loss of fluid. Thus this experiment would have to be considered inconclusive.

Since it was not feasible to give the cysts close microscopic scrutiny before removing them from the shrimp, it could not be ascertained whether metamorphosis of some had already occurred. Some premolt shrimps were taken very close to the oncoming molt. In these cases trophonts excysted during the dissections. It seems quite likely that metamorphosis had occurred before dissection was begun in such cases. It is also reasonable that the events leading to metamorphosis had already started for the other phoronts that excysted as trophonts. This excystation of trophonts is common when dealing with premolt shrimps. The unexpected result was the reappearance of the tomites.

However this reappearance of tomites does provide the evidence hitherto lacking for the independence of metamorphosis and excystation. Chatton and Lwoff (1935) imply from their discussions on metamorphosis of *Synophrya* that metamorphosis and excystation are in fact two different processes. Phoronts of apostomes have never been shown to excyst as tomites at any time before. Therefore, the readiness of the phoront to excyst under stress, and the reappearance of the tomite from the phoretic cyst were completely unexpected.

Even though the excysted tomites were not observed to settle on new hosts, their behavior was like that of tomites seeking a host in the normal course of their life cycle. Probably the tomite has only enough reserves to settle and encyst one time. Reserves remaining in the phoront are probably used for excystation. Since the tomite can not feed, its reappearance in this form is fatal to it.

In the course of this work no phoronts have been seen on the molt of the shrimp. Nor were empty cysts observed on intermolt shrimps. Apparently the normal life cycle proceeds without accident unless experimentally altered. But the results of this series of experiments establish that the ability to excyst is maintained throughout the phoretic stage.

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Summary

Pieces of exoskeleton bearing *Hyalophysa* phoronts from the bases of the eye-

stalks of intermolt and premolt *Palaemonetes* were placed in antibiotic brackish-water solution containing 0.05 M Tris-HCl buffer at pH 9.0, 0.5% glycogen, 0.5% β-D glucose, 0.5% N-acetylglucosamine, and 10^{-7} M ecdysterone. Controls were placed in antibiotic brackish water alone. In all experiments, the phoronts showed the same rates of excystation in control and experimental solutions. The phoronts excysted as tomites from intermolt shrimps, while both trophonts and tomites excysted from premolt shrimps. Experiments using body fluids from the host shrimp have indicated that the substance causing this unexpected excystation of tomites is in the body fluids of the host.

The resulting excystation of tomites from the phoretic cysts of *Hyalophysa* establishes the separation of metamorphosis and excystation in apostome ciliates.

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